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The reduced insulin-mediated glucose oxidation in skeletal muscle from type 2 diabetic subjects may be of genetic origin—evidence from cultured myotubes

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Abstract

Several defects in response to insulin have been described in vivo and in vitro in type 2 diabetes: a decreased glucose transport, defective glucose oxidation and altered glycogen synthesis. At present, it is unknown whether glucose oxidation is primarily affected or secondarily affected by, e.g. increased free fatty acids (FFA). The aim of this study was to evaluate whether myotubes established from type 2 diabetic subjects express a primarily or a FFA-induced reduced insulin-mediated glucose oxidation. We have therefore investigated glucose oxidation under basal, physiological conditions and during acute insulin stimulation with/without FFA. We found that myotubes established from type 2 diabetic subjects express a reduced insulin-stimulated increase in glucose oxidation. Moreover, an acute exposure to FFA reduces insulin-mediated glucose oxidation without alterations in glucose uptake and glycogen synthesis. Thus, we conclude that the diminished increase in insulin-stimulated glucose oxidation seen in type 2 diabetic subjects in vivo may be of genetic origin. Moreover, the glucose–fatty acid cycle seems not to be crucial for the pathophysiology of insulin resistance.

Keywords: Diabetes; Genetic; Glucose transport; Glycogen synthase; Glucose oxidation; Myotube; Skeletal muscle

1. Introduction

Type 2 diabetes mellitus is characterised by impaired insulin-mediated glucose metabolism. Several defects in response to insulin have been described in vivo and in vitro: a decreased glucose transport, defective glucose oxidation and altered glycogen synthesis [1–7].

The energy required by resting healthy muscles is obtained mainly from lipid oxidation and under conditions of insulin-stimulated metabolism shifted to an increased glucose oxidation due to the inhibition of lipolysis. During exercise, fat is the most important source of energy, but also glycogen is essential. This capacity to shift between fuels is designated metabolic flexibility. Subjects with type 2 diabetes or obesity seem to have a reduced lipid oxidation in the basal state and a reduced capacity to shift to glucose metabolism during insulin stimulation (metabolic inflexibility) (review Ref. [4]). In line with this, Stump et al. [8] showed that insulin stimulation has no effect on muscle ATP production for patients with type 2 diabetes, whereas non-diabetic controls increased their production with 16–26%. Recently, we have shown that lipid oxidation is primarily reduced in myotubes from type 2 diabetic subjects with a concomitant increase in phospholipid accumulation [9]. The glucose oxidation rate has, however, not yet been compared in myotubes established from controls and from subjects with type 2 diabetes, and at present it is unknown whether basal or insulin-mediated glucose oxidation is primarily affected in diabetic myotubes. Furthermore, it is unknown to which extent glucose oxidation is secondarily affected by, e.g. increased free fatty acids (FFA). In accordance with Randle's glucose fatty acid cycle, an increased lipid exposure will be followed by an increased lipid oxidation and a reduced glucose oxidation and thereby diminish the importance of glucose oxidation. In this context, cultures of human myotubes offer an excellent model for performing studies under standardised conditions: First, satellite cultures

Abbreviations: FFA, free fatty acids; PA, palmitate acid

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express traits known from in vivo muscles [3,10]; second, they allow to differentiate between genetic and adaptive processes [1,2].

In order to gain further insight into the mechanisms underlying muscle insulin resistance, the aim of our study was to clarify whether glucose oxidation rates were primarily (genetically) affected in diabetic myotubes and whether acute exposure to palmitate acid reduced glucose oxidation in accordance with Randles glucose–fatty acid cycle (adaptive) in myotubes established from controls and diabetic subjects.

2. Methods

2.1. Materials

Dulbecco's modified Eagle's medium, fetal calf serum (FCS), Ultroser G, penicillin–streptomycin–amphotericin B and trypsin were obtained from Life Technology (Scotland, UK). 2-[^{3}H(G)]deoxyglucose (259.0 GBq/mmol), D-[^{14}C(U)]glucose (173.1 MBq/mmol), and [1-^{14}C]palmitic (2064.6 MBq/mmol) were purchased from Dupont, NEN™ Life Science Products, Boston, MA, USA. Protein assay kit was purchased from BioRad (Copenhagen, DK). Glycogen, palmitic acid (PA) and ECM-gel were purchased from Sigma Chemical Co. (St. Louis, USA). Insulin Actrapid was obtained from Novo Nordisk (Bagsvaerd, DK).

2.2. Human study subjects

Ten obese type 2 diabetic patients and 10 matched control subjects participated in the study (Table 1). Only sedentary male subjects were recruited. None of the diabetic patients had received insulin treatment. The patients had no diabetic complications except from simplex retinopathy. The control subjects had no family history of diabetes. All subjects gave written informed consent, and the local ethics committee of Funen and Vejle County approved the study.

2.3. Cell culture

Cell cultures were established as previously described [10,11]. In brief, muscle tissue was minced, washed and dissociated for 60 min by three treatments with 0.05% trypsin–EDTA. The harvested cells were pooled and FCS was added as a protease inhibitor. The obtained cells were seeded for upscaling on ECM-gel coated dishes after 30 min of preplating. Cell cultures were established in DMEM medium supplemented with 10% FCS, 50 U/ml penicillin, 50 μg/ml streptomycin, 1.25 μg/ml amphotericin B and 25 pmol/l insulin. After 24 h, cell debris and nonadherent cells were removed by changing the growth medium to DMEM supplemented with 2% FCS, 2% Ultroser G, 50 U/ml penicillin, 50 μg/ml streptomycin, 1.25 μg/ml amphotericin B and 25 pmol/l insulin. Cells were subcultured twice in 100-mm Petri dishes before final seeding. At 75% confluence, the growth medium was replaced by a basal medium (DMEM medium supplemented with 2% FCS, 50 U/ml penicillin, 50 μg/ml streptomycin, 1.25 μg/ml amphotericin B and 25 pmol/l insulin) in order to induce differentiation. The cells were cultured in humidified 5% CO_2 atmosphere at 37 °C and medium was changed every 2–3 days.

Table 1
Clinical characteristics

	Control, $n=10$	Type 2 diabetic, $n=10$
Age (years)	51.1 ± 2.0	50.4 ± 1.6
Body mass index (kg/m^2)	29.6 ± 0.9	31.1 ± 1.1
HbA1c (%)	5.1 ± 0.1	6.9 ± 0.5*
Fasting plasma glucose (mmol/l)	5.5 ± 0.1	10.3 ± 1.1*
Fasting serum insulin (pmol/l)	40 ± 5	69 ± 9*

Data represent mean (± S.E.).
*$P < 0.05$ for T2D compared to control.

3. Experimental design

Human myotubes established from controls and subjects with type 2 diabetes were allowed to differentiate under physiological conditions of insulin (25 pmol/l) and glucose (5.5 mmol/l) for 8 days. All myotube cultures were used for analysis day 8 after onset of differentiation. Myotubes were rinsed twice and incubated for 4 h with either glucose or glucose supplemented with 0.6 mmol/l palmitate acids. The doses and the accumulation time were selected in accordance with our previous study evaluating palmitate oxidation [9]. Corresponding values of glucose uptake, glucose oxidation and glycogen synthesis were determined as described in Analysis at an insulin concentration of 25 pmol/l (designated baseline) and during acute insulin stimulation (1 μmol/l).

4. Analysis

4.1. Glucose oxidation

Cells were cultured in 12.5-cm^2 flasks and differentiated as described above. Cultures were exposed to DMEM supplemented with 0.24 mmol/l fatty free albumin, 50 U/ml penicillin, 50 μg/ml streptomycin, 1.25 μg/ml amphotericin B, D-[^{14}C(U)]glucose (2.0 μCi/ml) and 25 pmol/l or 1 μmol/l insulin, respectively, in order to study basal and insulin-mediated glucose oxidation. To study the impact of acute exposure to palmitate, sister cultures were incubated under the abovementioned conditions supplemented with 0.6 mmol/l palmitate. Flasks were air-tightened with a rubber stopper. After 4 h, 300-μl phenyl ethylamine–methanol (1:1, v/v) was added with a syringe to a centre well containing a folded filter paper. Subsequently, 300-μl 1 M perchloric acid was added to the cells through the stopper tops by means of a

syringe. The flasks were placed for a minimum of 1 h at room temperature to trap labelled CO_2. Cell-free flasks (no cell controls) went through the same procedure to correct for unspecific CO_2 trapping.

4.2. Glycogen synthesis

Cells were grown and differentiated in 12-well plates as described above. Cultures were exposed to DMEM supplemented with 0.24 mmol/l fatty free albumin, 50 U/ml penicillin, 50 μg/ml streptomycin, 1.25 μg/ml amphotericin B, D-[^{14}C(U)]glucose (1.0 μCi/well) and 25 pmol/l or 1 μmol/l insulin, respectively in order to study basal and insulin-mediated glucose uptake. To study the impact of acute exposure to palmitate, sister cultures were incubated under the abovementioned conditions supplemented with 0.6 mmol/l palmitate. The reaction was stopped after 4 h by aspirating the reaction mixture and rapidly rinsing each well four times with PBS at 4 °C. Measurement of glycogen synthesis using this method is linear up to 6 h (data not shown). Cells were solubilised by adding 0.5-ml 1.0 M KOH and afterwards heated at 70 °C for 20 min. To the sample was added 100-μl saturated Na_2SO_4, 100-μl 25 mg/ml glycogen in distilled water (freshly made) and 9-ml ice-cold absolute ethanol, and then left at −70 °C for 48 h for glycogen precipitation. The tubes were centrifuged (2000 × *g*, 20 min, 4 °C) and the supernatant immediately removed and discarded. The glycogen precipitate was redissolved in 500-μl distilled water by heating at 70 °C for 10 min, 9-ml ice-cold absolute ethanol was added and then left at −20 °C for a minimum of 24 h. Again, the tubes were centrifuged, the supernatants removed and 500-μl distilled water was added and the glycogen dissolved. Five-hundred microliters was removed for scintillation counting [12].

4.3. Glucose uptake

Cells were grown and differentiated in 12-well plates as described above. Cultures were exposed to DMEM supplemented with 0.24 mmol/l fatty free albumin, 50 U/ml penicillin, 50 μg/ml streptomycin, 1.25 μg/ml amphotericin B, 2-[^{3}H]deoxyglucose (0.2 μCi/well) and 25 pmol/l or 1 μmol/l insulin, respectively, in order to study basal and insulin-mediated glucose uptake. To study the impact of acute exposure to palmitate, sister cultures were incubated under the above mentioned conditions supplemented with 0.6 mmol/l palmitate. The reaction was stopped after 4 h by aspirating the reaction mixture and rapidly rinsing each well four times with PBS at 4 °C. Measurement of glucose uptake using this method is linear up to 6 h (data not shown). Cells were solubilised by adding 0.5-ml 0.1 M NaOH. An aliquot of 50 μl was removed for protein determination. The remaining fluid was placed in a scintillation vial, and scintillation fluid was added. Glucose transport activity is expressed as moles of 2-deoxy-glucose taken up per minute per milligram of total protein.

4.4. Statistical analysis

Data in text, tables and figures are given as mean ± S.E. Statistical analyses were performed using INSTAT 2.01 (GraphPad, USA). Students' *t*-test was used for unpaired and paired *t*-test for paired comparisons. $P \leq 0.05$ was considered significant.

5. Results

5.1. Insulin-mediated glucose oxidation is reduced in diabetic myotubes (Fig. 1)

We determined the baseline and insulin-stimulated glucose oxidation rates in myotubes established from type 2 diabetic and control subjects at 5.5 mmol/l glucose. Baseline glucose oxidation showed no significant difference between diabetic and control myotubes (382 ± 42 vs. 429 ± 41 pmol/mg/min, $P > 0.59$) (Fig. 1a). Glucose oxidation was sensitive to insulin stimulation in myotubes established from controls

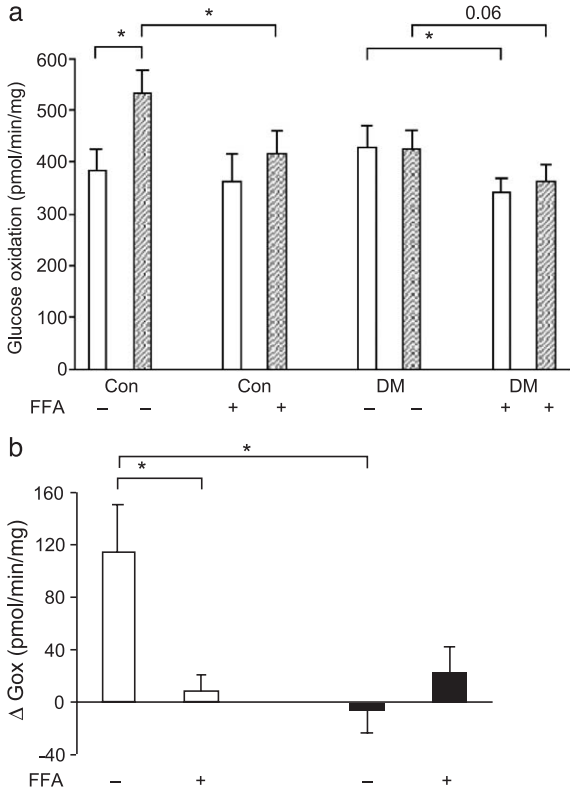

Fig. 1. Effect of insulin and/or palmitate acids on glucose oxidation in myotubes established from controls and type 2 diabetic subjects. Fully differentiated myotubes were acutely exposed to insulin (1 μmol/l) and/or palmitate acids (0.6 mmol/l) for 4 h. (a) Basal conditions (open bars), insulin-stimulated conditions (hatched bars). Data are means + S.E.; $n = 10$ subjects in each group. (b) The absolute differences between the basal and the insulin-stimulated glucose oxidation (ΔG_{ox}) in control (open bars) and diabetic cultures (black bars). Results are shown as means ± S.E., $n = 10$ subjects in each group. *$P < 0.05$ vs. corresponding basal condition or as indicated by bars. C = control, DM = type 2 diabetes, PA = exposure to palmitate acids.

(531 ± 46 vs. 382 ± 42 pmol/mg/min, $P < 0.05$), but not in myotubes established from subjects with type 2 diabetes (423 ± 38 vs. 429 ± 41 pmol/mg/min, $P > 0.54$). Insulin-stimulated glucose oxidation was significantly higher in control myotubes compared to diabetic myotubes (531 ± 46 vs. 423 ± 38 pmol/mg/min, $P < 0.05$). In order to gain further insight into the differences between diabetic and control myotubes during acute insulin stimulation, we compared the absolute differences between the basal and the insulin-stimulated states (ΔG_{ox}). The ΔG_{ox} for acute insulin stimulation was significantly higher in the control myotubes compared to myotubes established from type 2 diabetic subjects (114 ± 37 vs. −5 ± 19 pmol/mg/min, $P < 0.004$) (Fig. 1b).

5.2. Insulin-stimulated glycogen synthesis was reduced in diabetic myotubes (Fig. 2)

The glycogen synthesis rate in myotubes established from diabetic and control subjects on day 8 is shown in Fig. 2. No significant difference of baseline glycogen synthesis was found between diabetic and control myotubes (68 ± 12 vs. 50 ± 8 pmol/mg/min, $P > 0.05$). The glycogen synthesis rate during acute insulin stimulation was significantly lower in diabetic myotubes compared to control myotubes (141 ± 22 vs. 87 ± 12 pmol/mg/min, $P < 0.02$). However, acute insulin stimulation significantly increases the glycogen synthesis rate in both control (141 ± 22 vs. 68 ± 12 pmol/mg/min, $P < 0.01$) and diabetic myotubes (87 ± 12 vs. 50 ± 8 pmol/mg/min, $P < 0.05$) (Fig. 2).

5.3. Glucose uptake was insulin sensitive but with no differences between groups (Fig. 3)

We determined the glucose uptake at a glucose concentration of 5.5 mmol/l in control and diabetic myotubes. No significant difference of baseline (2.37 ± 0.08 vs. 2.12 ± 0.15 nmol/min/mg, $P > 0.32$) or insulin-mediated (2.85 ± 0.14 vs. 2.52 ± 0.16 nmol/min/mg, $P > 0.32$) glucose uptake was found between diabetic and control myotubes (Fig. 3). Control myotubes (2.85 ± 0.14 vs. 2.37 ± 0.08 nmol/min/mg, $P < 0.02$) and myotubes established from type 2 diabetic subjects (2.52 ± 0.16 vs. 2.12 ± 0.15 nmol/min/mg, $P < 0.01$) were sensitive to insulin stimulation (Fig. 3).

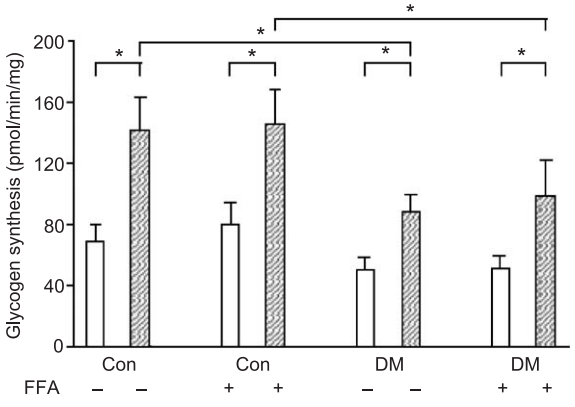

Fig. 2. Effect of insulin and/or palmitate acids on glycogen synthesis in myotubes established from controls and type 2 diabetic subjects. Fully differentiated myotubes were acutely exposed to insulin (1 μmol/l) and/or palmitate acids (0.6 mmol/l) for 4 h. Basal conditions (open bars), insulin-stimulated conditions (hatched bars). Data are means + S.E.; $n = 10$ subjects in each group. *$P < 0.05$ vs. corresponding basal condition or as indicated by bars. C = control, DM = type 2 diabetes, PA = exposure to palmitate acids.

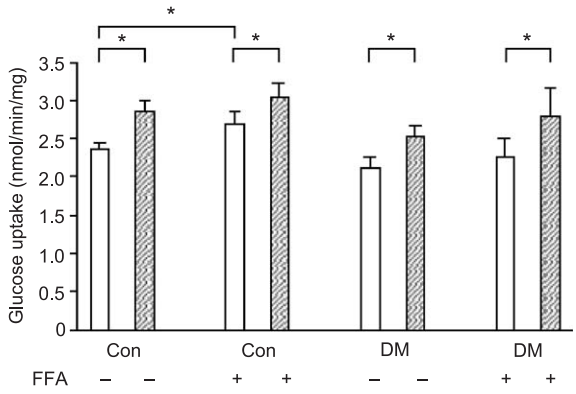

Fig. 3. Effect of insulin and/or palmitate acids on glucose uptake in myotubes established from controls and type 2 diabetic subjects. Fully differentiated myotubes were acutely exposed to insulin (1 μmol/l) and/or palmitate acids (0.6 mmol/l) for 4 h. Basal conditions (open bars), insulin-stimulated conditions (hatched bars). Data are means + S.E.; $n = 10$ subjects in each group. *$P < 0.05$ vs. corresponding basal condition or as indicated by bars. C = control, DM = type 2 diabetes, PA = exposure to palmitate acids.

5.4. Impact of acute lipid exposure

In an attempt to evaluate the effect of acute lipid exposure on glucose metabolism in myotubes established from controls and type 2 diabetic subjects, we determined glucose oxidation, glucose transport, and glycogen synthesis in sister cultures under the above mentioned conditions supplemented with 0.6 mmol/l palmitate.

5.5. Acute lipid exposure decreases insulin-mediated glucose oxidation in control myotubes (Fig. 1)

Baseline glucose oxidation was significantly reduced when diabetic myotubes was exposed to palmitate ($P < 0.05$). In contrast, control myotubes expressed no significant changes in baseline glucose oxidation when exposed to palmitate ($P > 0.05$) (Fig. 1). Acute lipid exposure decreases insulin-mediated glucose oxidation in control myotubes ($P < 0.05$) (Fig. 1), but not in diabetic myotubes ($P = 0.06$). ΔG_{ox} for acute insulin stimulation was reduced when exposed to palmitate in control myotubes (114 ± 37 vs. 8 ± 13 pmol/mg/min, $P < 0.05$), but not in diabetic myotubes (−5 ± 19 vs. 22 ± 20 pmol/mg/min, $P > 0.8$).

5.6. Glycogen synthesis in diabetic myotubes and control myotubes was not affected by acute palmitate exposure (Fig. 2)

Baseline and insulin-stimulated glycogen synthesis were not affected by acute exposure to palmitate acid in control ($P>0.05$) and type 2 diabetic myotubes ($P>0.05$) (Fig. 2).

5.7. Acute palmitate exposure and glucose uptake (Fig. 3)

Baseline glucose uptake in control myotubes, but not in diabetic myotubes, expressed a significant increase in glucose uptake ($P<0.05$) under acute lipid exposure. Insulin-stimulated glucose uptake was not hampered by acute lipid exposure neither in control ($P<0.05$) nor in diabetic myotubes ($P<0.05$). Insulin-stimulated glucose uptake was not significantly different between diabetic and control myotubes ($P>0.60$).

6. Discussion

The present study demonstrates that diabetic myotubes express a reduced insulin-mediated glucose oxidation and that acute FFA exposure hampers the insulin-mediated increase in glucose oxidation in control myotubes, but without reducing glucose uptake and glycogen synthesis. Thus, the diminished increase in insulin-stimulated glucose oxidation seen in type 2 diabetic subjects in vivo may be of genetic origin. Furthermore, the glucose–fatty acid cycle seems not to be crucial to the pathophysiology of insulin resistance.

Previously, it has been described that subjects with type 2 diabetes seem to possess an increased basal glucose oxidation and a reduced capacity to increase glucose oxidation during insulin stimulation (review Ref. [4]), but the mechanism responsible for this has not yet been identified. Whether the observed changes represent adaptive compensation at the cellular level or the direct expression of a primary genetic trait remains uncertain. In this context, cultures of human myotubes offer excellent material for performing studies under standardised conditions. The underlying idea when using myotubes is that myotubes only express their genetic background when precultured under physiological conditions and their adaptive traits when precultured under "inducing conditions". A genetic determination of known in vivo pathophysiological abnormalities will be evident if the abnormality is present in vitro under physiological conditions. Our study showed that the baseline glucose oxidation remained unchanged and insulin-stimulated glucose oxidation was significantly reduced in myotubes established from type 2 diabetic subjects compared to myotubes established from control subjects. This suggests that the increased basal glucose oxidation in diabetic subjects in vivo is an adaptive reaction, whereas the reduced glucose oxidation during insulin stimulation (review Ref. [4]) in diabetic subjects is of genetic origin. The inducing mechanisms responsible for this increased basal glucose oxidation in diabetic subjects are at present unclear.

Our observation of an impairment of insulin-stimulated glucose oxidation in diabetic myotubes is in line with the very recent work from Stump et al. [8] showing that insulin stimulation has no effect on muscle ATP production in patients with type 2 diabetes, whereas non-diabetic control subjects increased their production with 16–26%. Based on our findings, this reduced capacity to increase ATP production in type 2 diabetes seems to be a primary defect. Moreover, our study shows that the reduced insulin-mediated glucose oxidation is based neither on a concomitant reduced glucose uptake nor on an increased lipid oxidation [9]. Several studies have described a reduced expression of oxidative enzymes and activity [13–15] and, recently, Kelly et al. [16] described that both obese and diabetic subjects express mitochondrial dysfunction. In our study, myotubes were established from obese and obese with type 2 diabetes. Therefore, obesity per se or mitochondrial dysfunction due to obesity cannot explain all our findings. Moreover, both an affection of lipid oxidation [9] and insulin-mediated glucose oxidation without affection of baseline glucose oxidation imply that these oxidative defects in diabetic myotubes cannot be explained by a simple reduction in mitochondrial number or function.

The key regulator of glucose oxidation is pyruvate dehydrogenase (PDH), which is sensitive to insulin stimulation [17]. PDH activity is regulated by insulin-sensitive PDH phosphatase (activating PDH) and PDH kinase (inactivating) (PDK), which can be activated allosterically by acetyl-CoA, NADH and ATP. Mammalian tissues express four PDK isoenzymes [18] and two pyruvate dehydrogenase phosphatase isoenzymes [19]. Especially PDK4 has been interesting as the expression and activity are increased in rats with streptozotocin-induced diabetes which is not completely restored back to the level of control rats during insulin stimulation [20]. It could be speculated that the PDK4 activity is increased in diabetic myotubes either as increased expression or increased allosteric activation, thereby reducing glucose oxidation in diabetic myotubes. However, further studies are needed to test whether this hypothesis could explain primarily reduced insulin-mediated glucose oxidation in diabetic myotubes/subjects.

The well-described defect in the pathophysiology of type 2 diabetes is reduced insulin-mediated glycogen synthesis in skeletal muscles [1,2,7]. In line with previous studies, we showed that insulin-stimulated glycogen synthesis is reduced in diabetic myotubes compared to control myotubes, hence confirming that insulin-stimulated glycogen synthesis seems primarily affected in muscular insulin resistance. The observation that insulin-mediated glycogen synthesis and glucose oxidation are primarily affected emphasises that the impact of diabetes could be limited to the insulin effect. However, we recently showed that palmitate oxidation in diabetic myotubes seems primarily affected under baseline

conditions [9], thus indicating that insulin resistance may rather be seen as a changed metabolic setting.

The following question arises: why is glucose taken up and not stored as glycogen or oxidised to the same extent in diabetic myotubes as seen in control myotubes? This may be explained by the following: The MIRKO mouse, with muscle specific inactivation of the insulin receptor gene [21], expresses a reduced insulin-stimulated glucose transport and glycogen synthesis with a concomitant threefold increase in insulin-stimulated glucose transport in adipose tissue, thus indicating that selective muscular insulin resistance redistributes glucose to adipose tissue [22]. In accordance with the MIRKO mice, it could be speculated that these primary defects in myotubes established from type 2 diabetic subjects are responsible for the redistribution of glucose to other tissues, e.g. adipose tissue. This redistribution may add to the development of obesity as seen in the MIRKO mouse.

Previous studies have shown that the capacity to take up glucose under basal conditions and during acute insulin stimulation is reduced in diabetic myotubes [3,23], but the insulin responsiveness is conserved [1,3,23]. No difference was found in glucose uptake in myotubes established from insulin-resistant and insulin-sensitive first-degree relatives [24]. In line with this, the present study confirms a conserved insulin responsiveness in diabetic myotubes, increasing the evidence that the glucose transport does not seem to be primarily insulin-resistant in diabetic myotubes. In human skeletal muscles, most of the glucose taken up and mediated by insulin is stored as glycogen [25]. In contrast, only 9–16% of insulin-mediated glucose uptake in myotubes is stored as glycogen, thus implying that even when glycogen is primarily affected it would be impossible to detect an impact on glucose uptake. However, the identical glucose uptake flux ensures that the described primary defects in glucose oxidation and glycogen synthesis are not based on differences in glucose uptake.

Several in vivo studies have found that increased palmitate exposure is followed by an increased lipid oxidation and a reduced glucose oxidation, but glucose disposal was only reduced after former pathways had been affected, thus indicating that Randle's cyclus is functionally operating, but not responsible for insulin resistance [26,27]. In line with this, we showed that acute palmitate exposure was followed by a hampered insulin-mediated glucose oxidation in control myotubes, but not in glucose uptake or storage. By choosing a 4-h exposure at a 0.6 mmol/l palmitate concentration, we separated the palmitate effects on glucose oxidation from those on glucose transport and glycogen synthesis, thus allowing us to study the effect of palmitate on glucose oxidation. Although neither reduced glucose transport nor glycogen synthesis was detected in our cultured myotubes exposed to palmitate, several studies have shown that glucose transport and glycogen synthesis are reduced when the cells are exposed to increasing levels of FFA for longer periods of time [28,29]. The finding that acute palmitate exposure hampered the insulin-mediated increase in glucose oxidation in control myotubes may ensure that, under conditions of active lipolysis, glucose is not oxidised in muscles. The responsible mechanism may be the increasing palmitate level rising the acetyl-CoA level and thereby allosteric inhibiting PDH. We studied the effect of a specific fatty acid, palmitate, but other fatty acids (unsaturated or essential fatty acids) may have other effects on the metabolism than secondary insulin resistance on glucose oxidation.

In conclusion, our data suggest that myotubes established from type 2 diabetic subjects express an inborn reduced insulin-mediated glucose oxidation and glycogen synthesis—both may be a part of insulin resistance in type 2 diabetes. Furthermore, acute exposure to palmitate induced insulin resistance at the glucose oxidation level. Although acute exposure to palmitate reduced glucose oxidation, glucose transport was not reduced, thus suggesting that Randle's cyclus is not a part of the pathogenesis of insulin resistance.

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